Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• Membrane test (Dialysis tubing on top of an agar plate) • Prototype for transdermal patches		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body	• 3/25 collect the membrane tests
Andrew	• Liquid culture of UV promoter for miniprep			• Miniprep for sequencing

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan	• Collected the membrane test	• One leaked bacteria (not sterile enough) • One didn’t leak	• We've concluded that the experiment isn't sterile enough • Two tubes had contradicting results	• Redo with liquid membrane test
Bryan	• Liquid membrane test • Dialysis tube inserted into a 50 ml centrifuge tube		• Liquid membrane test could have a better result than the plate test because it has a greater surface area in contact with the LB • If any bacteria leaks, it will grow in the LB	• 3/26 pickup the liquid membrane test
Andrew	• Mini-prepped UV Promoter for sequencing • Liquid culture of UV promoter		• Finished UV promoter PP for sequencing	• Running another protocol for UV mini-prep sequencing

Experiments

Thursday, April 16, 2015
12:30 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Prototype Testing	Bryan	• Collected the 50 ml centrifuge tube		• The experiment wasn't sterile enough • The tube had bacteria both inside and outside	• 4/7 Redo Liquid plate test
Temp+ GFP Testing	Andrew	• Placing Temp + GFP plates into 42 degrees to see if the promoter works as described on the iGem website	• No GFP expression after 1h, leaving it overnight	• Designed an experiment to test the Temp + GFP construct • Experiment- Grow re-streaks of Temp+ GFP at Room Temp, 37 Degrees and 42 Degrees	• Ran experiment as described in conclusion • Going to check on 3/27

Experiments

Thursday, April 23, 2015
5:01 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Check if Temp+ GFP construct works	Andrew	• Checked re-streaks of Temp + GFP that has been grown overnight at RT, 37 Degrees and 42 Degrees	• RT did not grow • Both 37 and 42 degrees grew and had GFP expression	• Expression at 32 Degrees is problematic • Redesigning experiment	• Grow Re-streaks of Temp + GFP at 32 and 37 degrees to see which one allows bacteria to grow well while not expressing GFP
Checking Dialysis tube experiment	Andrew	• Checked Dialysis tube experiment (50 ml tubes) • Checked Dialysis tube (agar plates)	• 50 ml tube setup seems to be contaminated • No colonies on plates	• 50 ml tube seems to be contaminated since the LB in it does not have any resistance	• Redo 50ml setup and leave the plates our during spring break to check if the plates will grow if given enough time

Weekly plan

Friday, April 17, 2015
8:03 PM

Group	Goal
Monica + Joseph	• Finish temperature sensitive promoter (front insert) + GFP gen

Outside of class schedule

Friday, April 17, 2015
8:03 PM

Group	Date	Time (e.g. period, after school, etc.)	Experiment
Monica + Joseph	4/20	A3	• Streak + liquid culture temp sens promoter + GFP gen in different temperatures

Weekly summary

Friday, April 17, 2015
8:03 PM

What we accomplished:	
What we need to do:	

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Evonne	• Agar plate test • Liquid culture (RFP + UV) for new dialysis tube testing		• Preparing for the 4th prototype	• 4/14 collect the liquid culture
Bryan	• LB testing • Liquid culture (RFP + UV) for new dialysis tube testing		• Preparing for the 5th prototype	• 4/14 collect the liquid culture
Andrew	• Digested Temp at ES • Did gel check of digestion		• Passing on Temp + GFP testing to Joseph + Monica	• Gel purification of Temp, ligation + transformation

Experiments

Friday, April 17, 2015
3:21 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

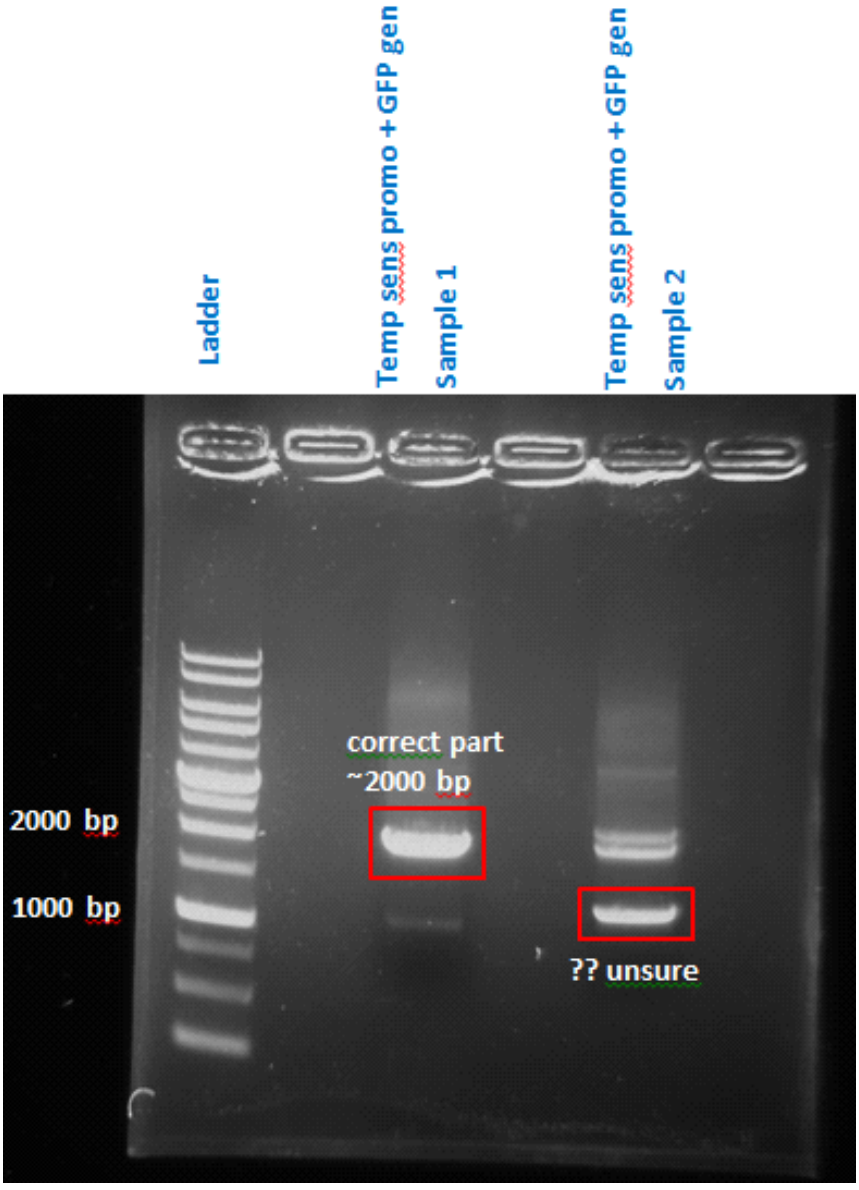
Joseph + Monica	• Gel check for temp sens promoter + GFP gen
	  • Picked 2 colonies from plate that glowed
	  • 2 colonies, 2 lanes
	• Expected size: ~2000 bp
	  • GFP gen: ~900 bp
	  • Promoter: ~900 bp
		• Band at ~2000 bp in both lanes
		• However, Lane 2 had a band at ~1000 bp
		• Gel pic **(Figure 1)**
			• Lane 1 showed successful ligation of temp sens promoter + GFP gen
			• Don't know what was the extra band in Lane 2 so only kept sample in Lane 1
				• Experiment with what temperatures bacteria would produce GFP at 4, 25, 30, 37, and 42 degrees C

Evonne and Bryan	• Collect liquid cultures			• 4/15 Set-up membrane tests
Andrew	• Ligation and transformation ofTemp+ RBS			• 3 in 1 of the Temp + RBS

Photos

Friday, April 17, 2015
3:23 PM

Figure 1. Temp sens promoter + GFP gen construct gel check



Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

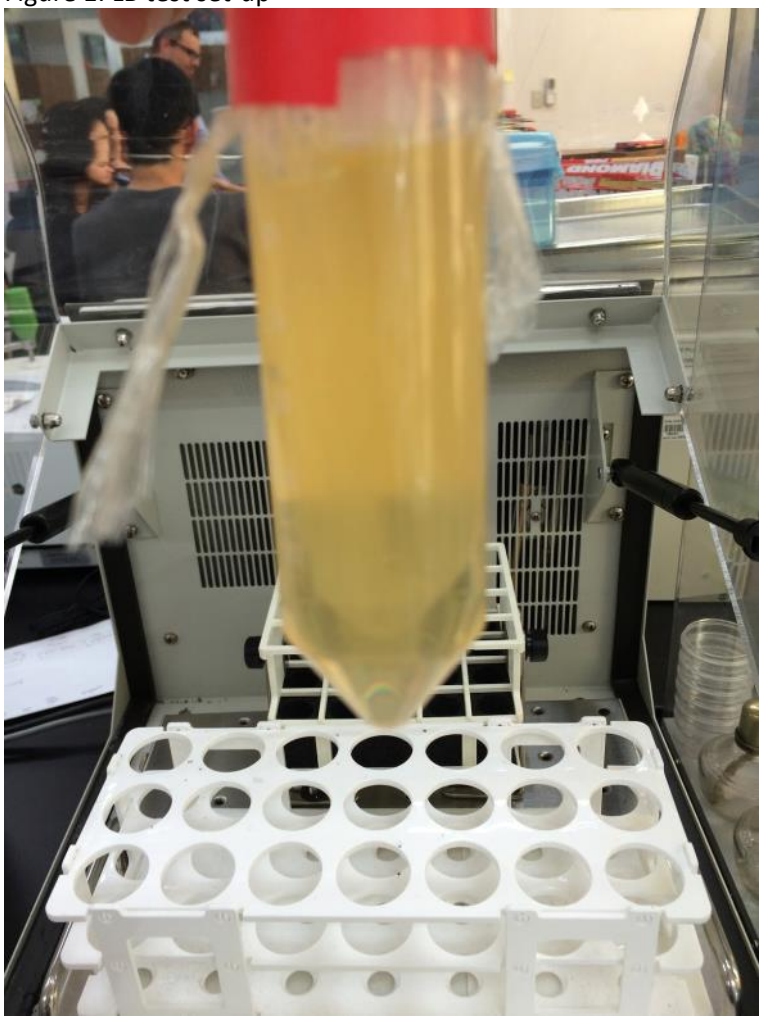
Joseph + Monica	• Planning for Temp Sens + GFP construct testing:
	  • Use OD600 to balance pop.
	  • Use 395nm as excitation, 509nm as emission for GFP
	  • Test groups: 4, Room, 30,37, 42 C
	  • Plate and tube for each
			• OD600 is to make sure that concentration of bacteria would be same in each cuvette sample as bacteria in the liquid culture would grow at different rates in different temperatures
			• Use 395 nm and 509 nm to measure GFP production
			• 30 degrees C = skin temperature
			  • Could be useful for drug delivery
				• 4/20: streak + liquid culture bacteria; place in different temperatures
				• 4/21: spectrovis

Evonne	• Membrane test (Dialysis tubing on top of an agar plate) • Prototype for transdermal patches • In contrast to the first prototype, the bacteria used has a UV promoter and RFP as a reporter • Two trials		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body • The 4th prototype must be more sterile	
Bryan	• Membrane test (Dialysis tubing within a 50ml centrifuge tube) • Prototype for transdermal patches • In contrast to the 3rd prototype, the bacteria used has a UV promoter and RFP as a reporter • Two trials		• The 5th prototype must be more sterile • Set-up --> see Figure 1	
Andrew	• Temp + RBS 3 in 1			• Run gel for PCR

Photos

Thursday, April 16, 2015
12:30 PM

Figure 1: LB test set-up



Within the dialysis tube: Liquid culture of UV + RFP
Outside the dialysis tube: LB

Experiments

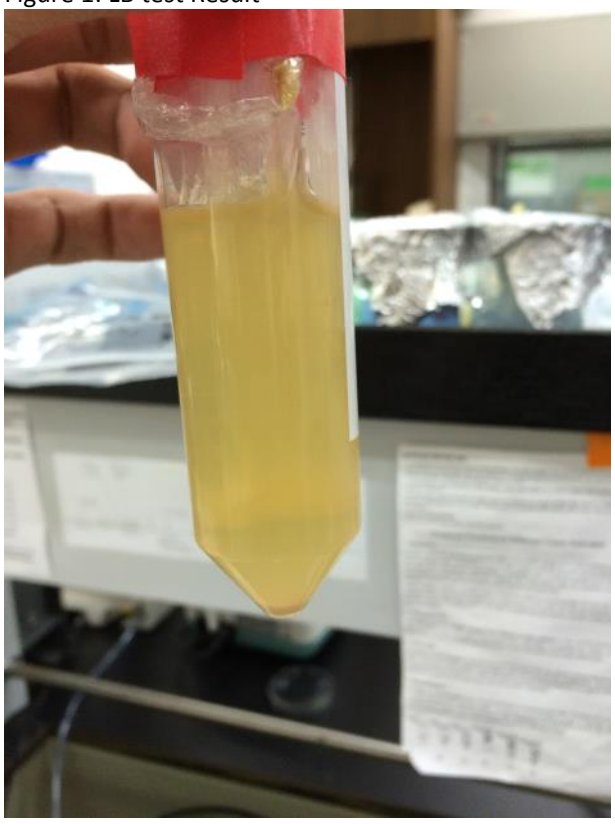
Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Evonne	• Collected the membrane test	• Both membrane tests had bacteria growing on the agar plate	• The testing was sterile but still had bacteria growing • Should revise the protocol and review mistakes that can occur during the experiment	4/23 Seek for further directions regarding membrane tests
Bryan	• Collected the membrane test	• Both membrane tests had bacteria growing outside the dialysis tube	• The testing was sterile but still had bacteria growing • Should revise the protocol and review mistakes that can occur during the experiment	4/23 Seek for further directions regarding membrane tests
Andrew	• Ran gel for Temp + RBS PCR • Liquid culture of temp	• Band is incorrect	• Need to re-digest RBS	• Mini-prep temp and digestion of both temp and RBS

Photos

Tuesday, April 21, 2015
3:36 PM

Figure 1: LB test Result



Bacteria grew both inside and outside the dialysis tube

Figure 2: Plate test Result

Bacteria grew outside the tube

Experiments

Thursday, April 23, 2015
5:40 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Andrew	• Mini prep of Temp • Digestion of RBS at EX • Digestion of Temp at ES • Ran Gel for digestion			• Gel digestion + Ligation + Transformation of Temp + RBS

Guidelines

Friday, April 17, 2015
2:21 PM

For each separate experiment, keep adding rows.

Figures should be fully labelled on program of your choice (has to be able to add solid text, arrows, shapes, and rotate those), saved as a jpg file, and uploaded to daily folders. Fully labelled = arrows identifying different parts, boxes pointing things out, text labelling plasmid sizes on gels, etc.

Group: who are involved in the experiments
Experiments: what did you do? include specific details like restriction sites, inserts, etc. note changes to protocols and should be specific to the point others can follow along what you did
Results: what were the results? what did you observe? what were some significant things to point out? if you say successful/unsuccessful explain what makes it so
Conclusions/notes: what do your results mean? propose a reason why the results turned out the way it did
Next steps: what are you going to do next and why?

2015年7月24日
下午 04:41

Summary

Thursday, April 23, 2015
4:18 PM

Temperature sensitive promoter
This week we focused on testing for the activation temperature of the temperature sensitive promoter. We grew plates at temperatures from 4 degrees C to 42 degrees C. We found that the promoter is activated at 37 degrees C. We could further this experiment by looking at the amount of production of GFP.

Mutagenesis
This week we finished the first point mutation of ACT. We completed PCR and miniprep for this and are ready to send it to sequencing to check if the point mutation was successful.

New UV promoter
This week we got the UV promoter back from sequencing and found that the promoter provided by the iGEM headquarter was a constitutive promoter rather than one activated only under UV light. Therefore, that explains for why the bacteria with UV promoter + RFP construct always produced RFP whether it was under the light or not. The iGEM headquarter sent us another package with the new UV promoter; however, it included the constitutive promoter too. Therefore, we are planning to send it back to sequencing to ensure that we got the correct promoter.

Temperature sensitive promoter + RBS
Last week we confirmed that the temperature sensitive promoter works. So this week we are building the promoter + RBS construct.

Experiments

Friday, April 17, 2015
3:21 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	Growing temperature test cultures - liquid and plates • 4*C, Room temp, 30*C, 37*C, 42*C			To check fluorescence for promoter expression
Mutagenesis	Leon	1. Transform ACT Muta 1 Trial 2 long protocol 5 min ice 30 second 42c 5 min ice 1000uL LB 60 min 37c 250 rpm plate all 2. LB Agar plate test prepared 100mL samples 6 groups: 1. 15 g/L 2. 17 g/L 3. 20 g/L 4. 22 g/L 5. 25 g/L 6. 30 g/L 3. grew liquid cultures for temp sens promoter 5mL LB + 5uL amp	1. Transform ACT Muta 1 Trial 2 - FAIL plates were inverted to incubate right side up no single colonies on plate 2. LB Agar plate test autoclaved and poured into large plates viscosity is obvious 3. temp sens promoter cultures all grew	1. salvage adding 500uL lb to lid and plate shake it around to pick up some bacteria re-plate to attempt to salvage single colonies 2. unsure yet, going to have to actually streak or transform some stuff will update in future 3. cultures still grow and resistance is correct	1. salvage tomorrow 2. testing in future updates in later days 3. miniprep for sequencing and keeping

Pictures

Thursday, April 23, 2015
8:22 AM

Experiments

Tuesday, April 21, 2015
3:34 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Mutagenesis	Phillip	Started reattempt at PCR for mutagenesis of PstI sites. This is our 3rd attempt for the first mutagenesis of the PstI site. The first attempt has turned out to be unsuccessful. I do not have any good ideas of why that is the case. We have made certain revisions to the PCR procedure and hopefully this will work. I also transformed ACT-A and ACT-B samples so that we may have more plasmid later on to purify	Will update tomorrow Plates: PCR: Need gel check tomorrow	Only time can tell.	Digestion and then transform at the end of the day. Hopefully, I will also be able to finish plasmid purification to have more ACT plasmids.
UV promoter	Paul and Dylan(Dylan is absent)	Today Paul helped get the new UV promoter and the constitutive promoter from the mail office. Afterwards Paul took the new promoters and had them streaked onto Ampicillin LB agar plates using inoculated loops (UV sterilized)	Paul has successfully streaked the promoters onto the plates, and Paul has also successfully retrieved the packages from the mail office.	Successful streaking of the plates, however the content of the mail package from the iGEM headquarters is slightly questionable because the iGEM headquarters sent wrong promoters along with the correct promoters. Therefore we have decided that we would have the promoters sequenced to make sure that they are of the correct kind. Just a small note the promoter that we are trying to test to see if it works in our potential construct is the UV promoter and is not the constitutive promoter that the IGEM headquarters keeps on sending to us.	Paul will come in tomorrow to come retrieve the plates that he streaked out today and he will put the plates into the refrigerator that is located under the lab bench. Afterwards the promoters will be shipped off for sequencing by a third-party contractor.
Testing temperature sensitive promoter	Daphne and Jon	Helped to streak temperature sensitive promoter. We labeled each plate with different temperatures and set three incubators according to those temperatures: 32 C, 34 C, 35C.	Successfully streaked the promoter onto the plates and placed into the incubators.	Results will be out tomorrow	Joseph will take over from now and check the results of whether if it glows or not tomorrow.
Mutagenesis	Leon	1. Checked on ACT Muta 1 Trial 2 transformation 2. setup hotplate temperature gradient incubation 3. autoclave + pour LB Agar plates 4. salvaged act Muta 1 trial 2 plates	1. ACT Muta 1 Trial 2 initially failed, waiting on salvage 2. seems to work, might use this setup for temperature gradient, unsure 3. plates are good, ready in fridge	1. not sure if m1t2 should be restreak salvaged 2. picture of set up in **(Figure 3)** 3. test on ACT stuff 4/22 , 4/23 4. this is a very sketch method added 500ul of lb to lid shook it a little poured into a new plate repeat	1. ACT Muta 1 Trial 2 is done 2. further tests 3. streak stuff to test plates 4. liquid culture + miniprep + sequencing?
Testing temperature sensitive promoter	Joseph Monica	1. Checked cultures for temp sense + GFP from yesterday 2. Measured population of liquid cultures with spectrovis	1. Cells did not grow at 4 degrees and RT 2. Cells grew at 30, 37 and 42 degrees **(Figure 1)** 3. Cells glowed at 37, 42 degrees **(Figure 2)** 4. Still doing spectroanalysis	1. Promoter activated somewhere between 30-37 degrees 2. Note: temperature was hard to maintain in black incubator (30 degrees)	1. New plates - from 34, 35 and 32 degrees to pinpoint temperature that promoters are activated at 2. Repeat procedure - may use data for modeling
Temp + RBS construct	Andrew	• Did gel digestion + ligation of Temp+RBS • Transformed the Temp RBS • Heat deactivated and saved ligation mix just in case			• Checking plates next day and 3 in1

Pictures

Wednesday, April 22, 2015
4:56 PM

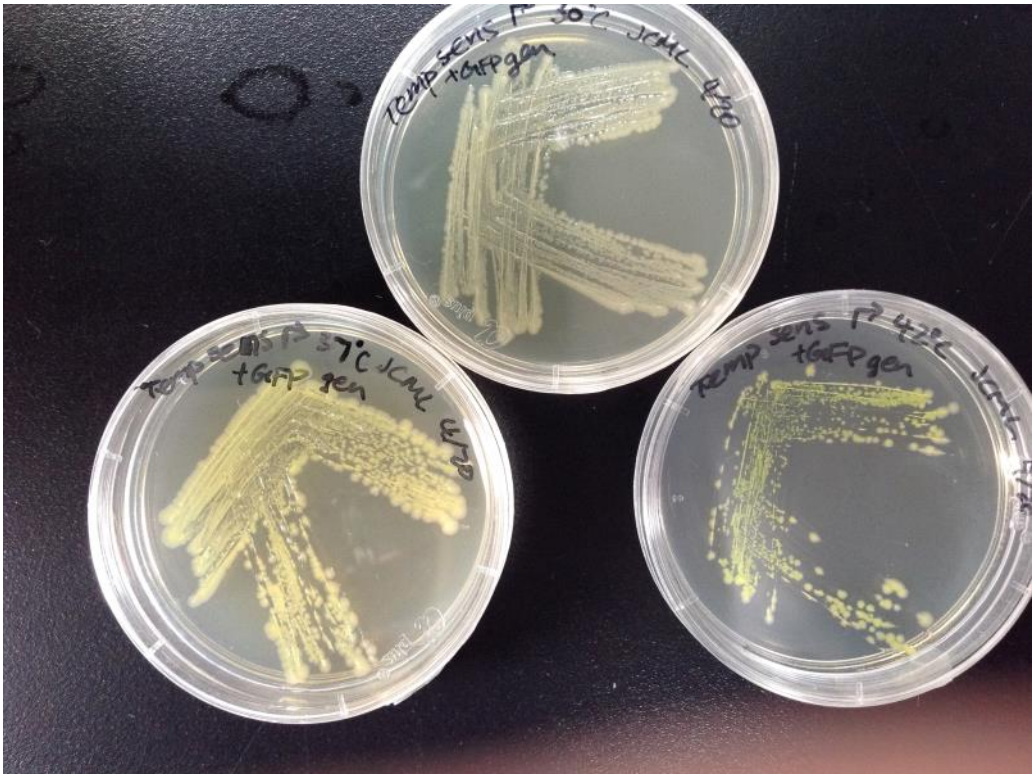


Figure 1. Bacteria carrying GFP generator driven by a temperature-sensitive promoter grow at 30, 36, and 42°C.

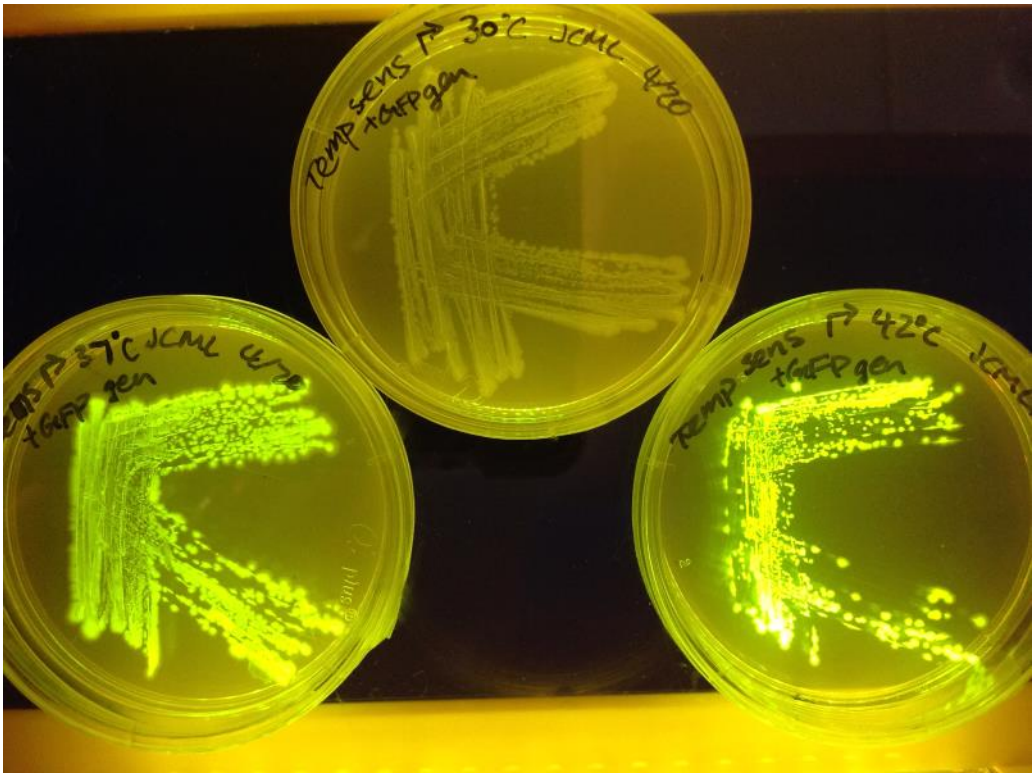


Figure 2. GFP generator is expressed at temperatures above 36°C.

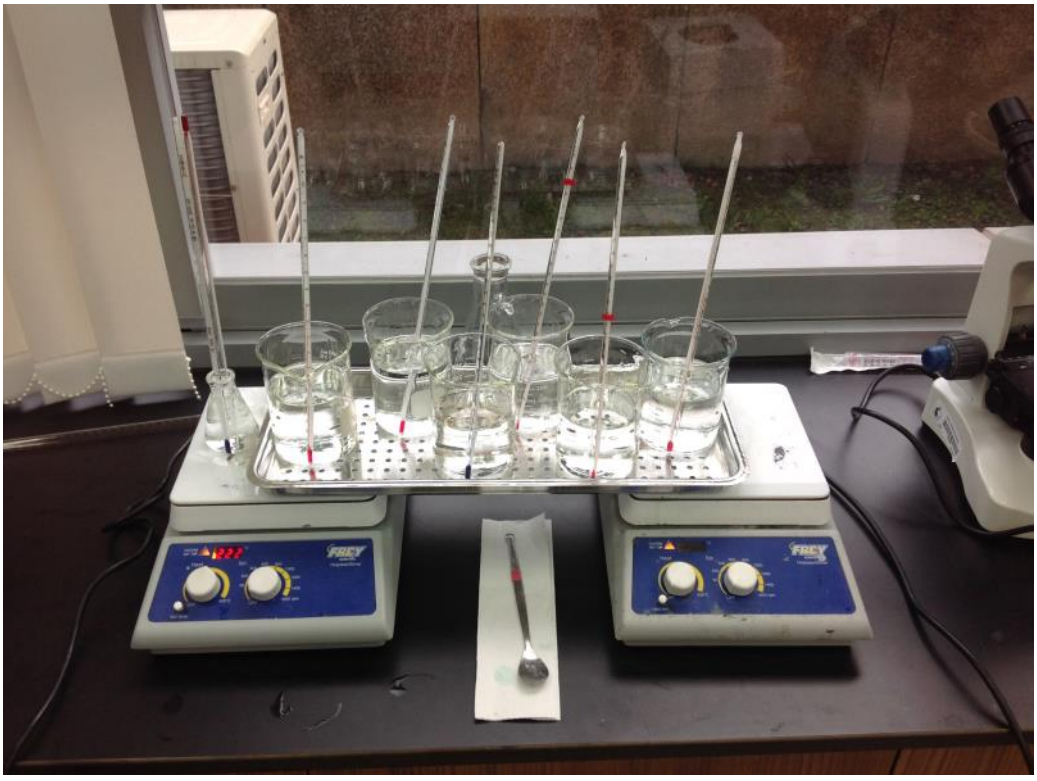


Figure 3. hotplate setup for multi temperature water bath incubation

Dialysis Tubing Tests

Tuesday, April 21, 2015
2:28 PM

Evonne and Bryan

1st membrane test (Plate):

March 24th, 2015

- Membrane test (Dialysis tubing on top of an agar plate)

March 25th, 2015 (PD Day)

- Collected the membrane test
    - One leaked bacteria (not sterile enough)
    - One didn't leak

2nd membrane test (LB)

March 25th, 2015 (PD Day)

- Made the liquid culture membrane test
    - Dialysis tube inserted inside a 50 ml centrifuge tube

March 26th, 2015 (PD Day)

- Pickup liquid culture membrane test

3rd membrane test (Plate)

April 7th, 2015

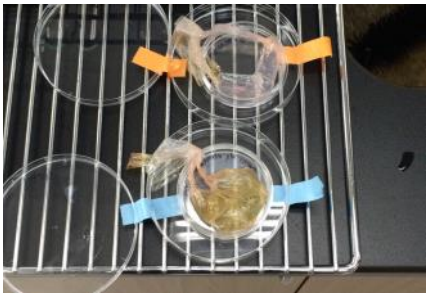
- Liquid Culture

April 9th, 2015

- Set-up membrane test for liquid culture

April 10th, 2015

- Both membrane tests dried out, one leaked



4th membrane test (Plate and LB)

April 13th, 2015

- Liquid culture (RFP + UV) for new dialysis tube testing
- Evonne - plate
- Me - liquid testing
    - More preferable because greater surface area

LB testing setup:
20ml Liquid culture in dialysis tube
15ml LB outside the dialysis tube

- If any bacteria leaks it's contaminated

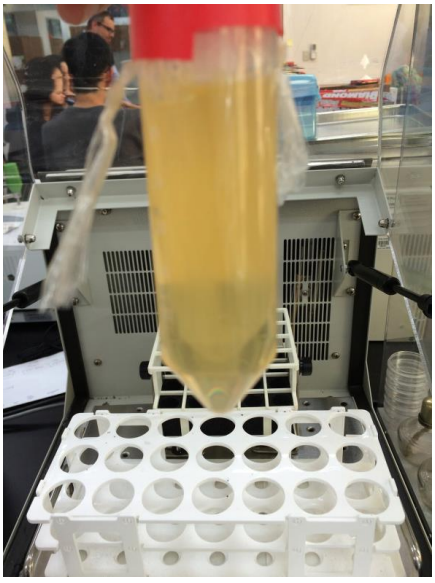
April 14th, 2015

- Pickup liquid cultures

April 15th, 2015

- Set-up membrane test

LB test:



April 16th, 2015

LB test:

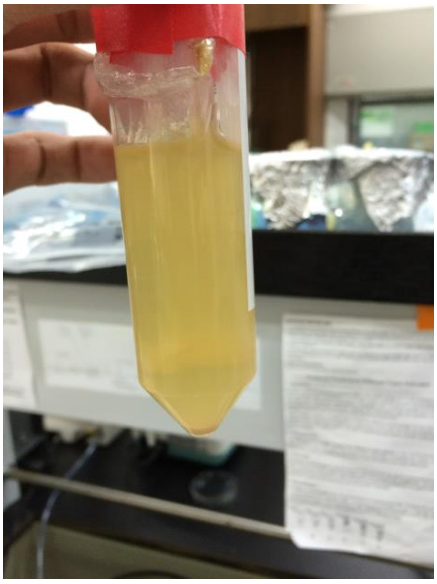


Plate:

Dr. Chiang said experiment failed.
Just because the plates showed red color does not mean that proteins passed through dialysis tube and bacteria didn't
Need to think of new method/membrane

Experiments

Tuesday, April 21, 2015
4:02 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
	Phillip				
Testing temperature sensitive promoter	Joseph, Monica	Plates failed - temperatures were altered some time Doing reruns at 30, 32, 35 and 37			
Testing UV promoter	Daphne	Finished 4 tubes of liquid culture (with amp)on the 2 plates of new UV promoter that was streaked by Paul on 4/21 I gave the tubes to Joseph who will put them into 37 degrees incubator for me.	Oops I used out all the small gloves	Update tomorrow	Come back tomorrow to proceed!!!!!!!!
Temp + RBS construct	Andrew	• Plates got contaminated- Redo • Mini-preped Temp promoters to restock PP			• Retransform ligation mix of Temp + RBS
ACT and ACT m1t3	Leon	• plates were wet and perhaps contaminated (figure 3,4) • M1t3 looks fine (figure 5,6)	no single colonies, bacteria smeared	if plates are wet then single colonies won't form	culture + restreak

Pictures

Tuesday, April 21, 2015
4:03 PM

Figure 1: This is a gel check. Without ladder because I loaded the PCR Mastermix instead. Oops

Figure 2: Plates for UV promoter

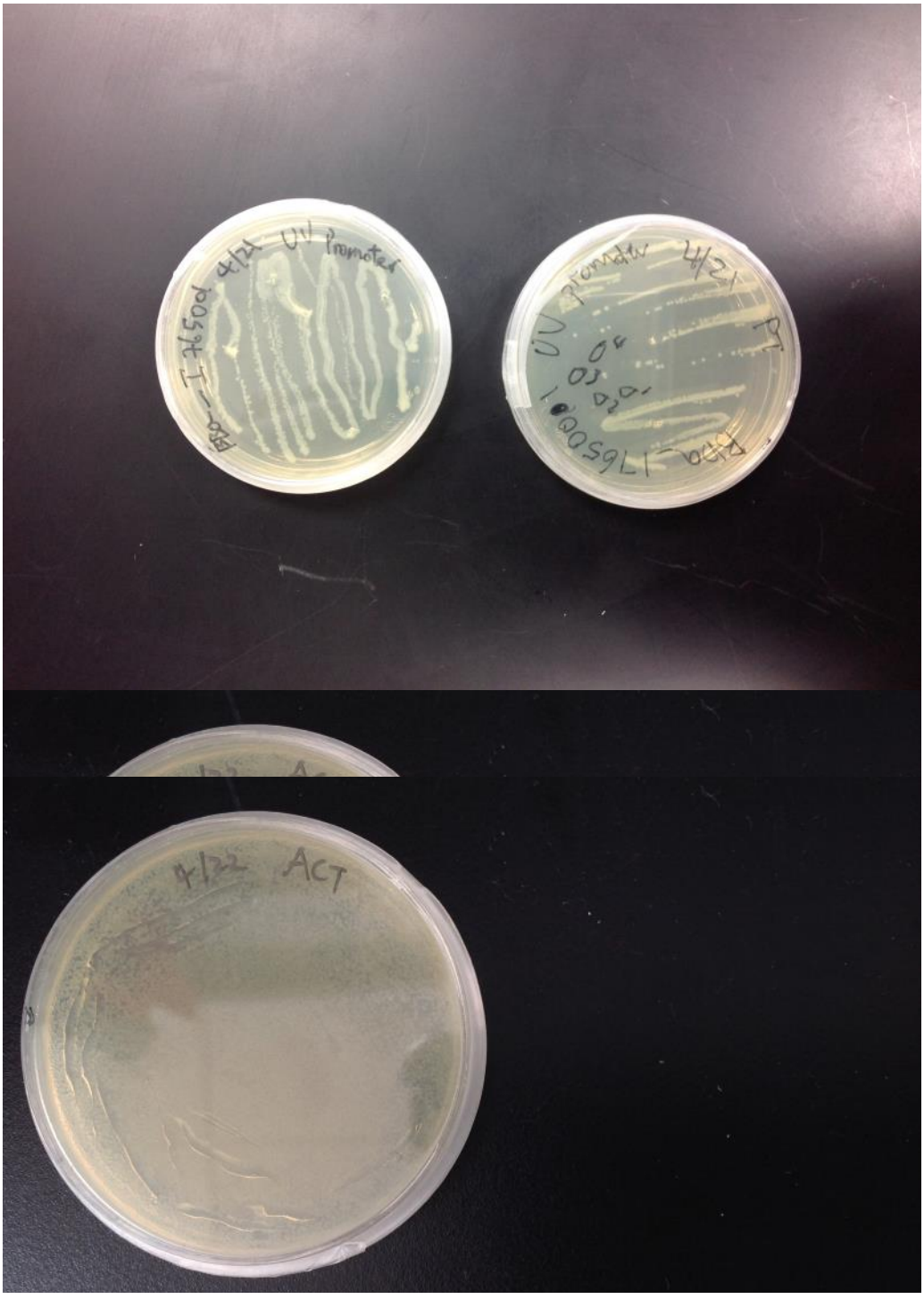


Figure 3 ACT transformation plate 1 smeared

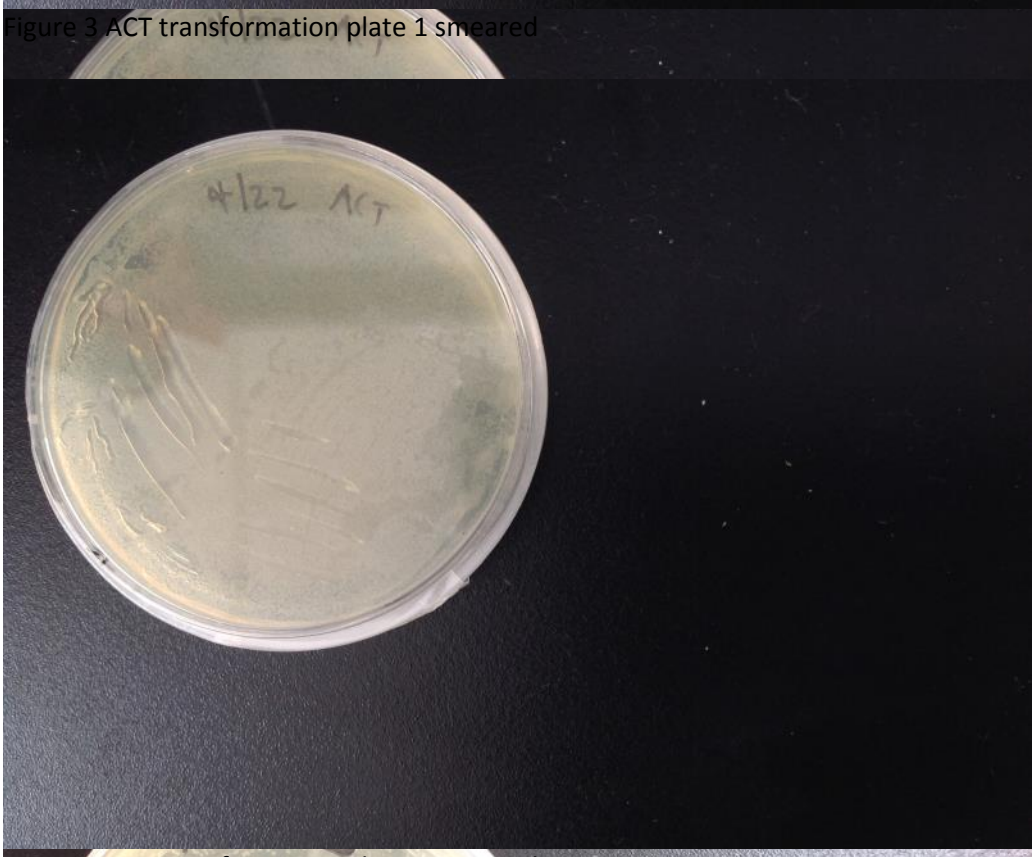


Figure 4 ACT transformation plate 2 smeared

Figure 5 ACT M1T3 B transformation: looks good

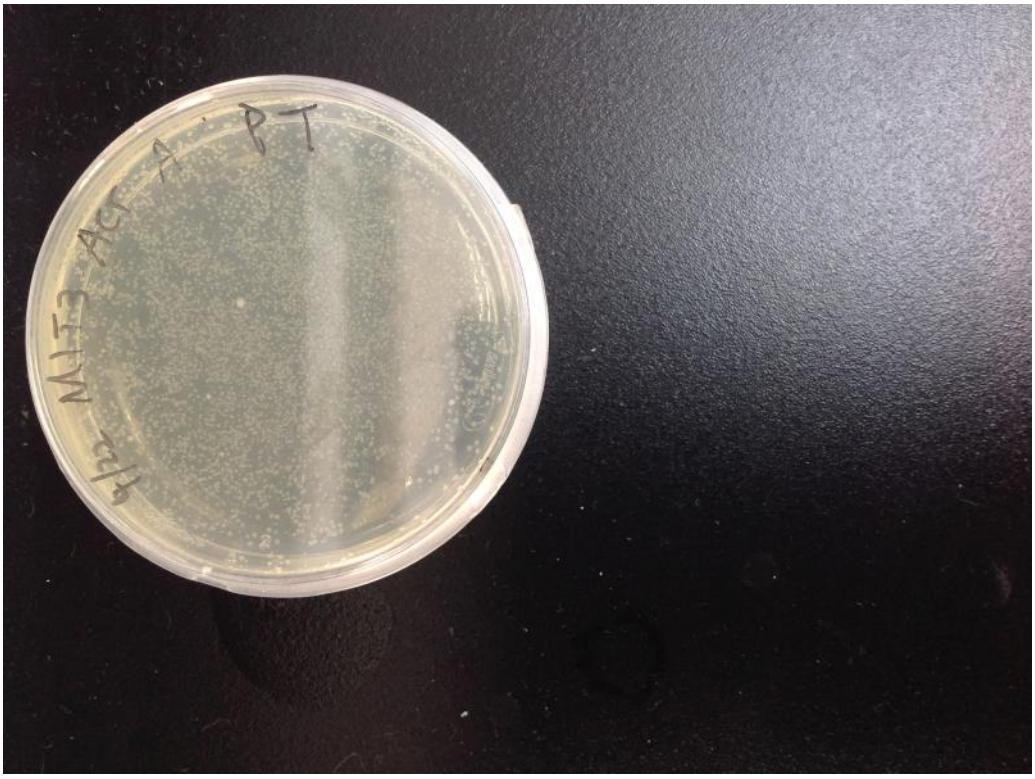


Figure 6 ACT M1T3 A transformation: looks good

Experiments

Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	1. Checked results for temp sens promoter + GFP gen at 30, 32, 35, 37 degrees C **(Figure 1 and 2)** 2. Streaked new plates for temp sens promoter + GFP gen at 35 and 36 degrees C	1. Colonies grew in all plates; however, only glowed at 37 degrees C	• We think that the activation temperature is somewhere in between 35 and 37 degrees C	• Check results for plates at 35 and 36 degrees C
Mini-prep of Temp Sens Promoter	Andrew	Mini Prep Temp Sens Promoter for sequencing			
Testing of human body temperature for the temperature sensitive promoter	Paul and Dylan with Dylan being absent	Took Mr. Tsao's laser temperature scanner and used it to take measurements of the community.	I collected the results and compiled them onto an excel spreadsheet on my Google doc folder.	No additional notes apart from collecting the body temperatures of various people in the school community.	Next step is to analyze the data that has been put into the Google Doc spreadsheet
Testing the new UV promoter	Daphne and Jon	Finished miniprep of Philip's 3 liquid cultures. Jon did 3 tubes (with WI and water)and Daphne did 3 tubes of miniprep using regular protocol.	Daphne finished miniprep, Jon's tubes added elution instead of water so need to be redone tomorrow.		Daphne's tubes move on to digestion and Jon redo miniprep • Digestion for testing • Miniprep for sequencing
Temp + RBS	Andrew	• Transformed ligation mix from 4/21			• Checking plates next day
Temp promoter sequencing	Andrew	• Mini-prepped Temp promoter with sequencing protocol			• Sending PP for sequencing

ACT M1T3	Leon	• streaked 7 plates for A
		  • varying concentration agar
		  ○ 15, 17, 20, 22, 25, 30 g/L
		• streaked 7 plates for B
		  • varying concentration for agar
		  ○ 15, 17, 20, 22, 25, 30 g/L
		• streaked 4 extra plates
		• grew cultures for m1t3 A and m1t3 B
			• all grew didn't keep all due to spatial constraints (figure 3,4,5,6,7,8,9,10)	• 20 g/L, 22 g/L and 25 g/L are most easily streaked	• restreak/lb
					• lb cultures for act dna
					• sequence to make sure it's correct

ACT	Leon	• streaked 12 big plates + 2 small plates
		  • big plates varying agar conc.
		  ○ 15, 17, 20, 22, 25, 30 g/L
			• all plates grew very well
			  • kept most of the plates for a restreak
				• bacteria are happy and growing on amp plates
				• agar concentration does not inhibit growth
					• restreak single colonies

Pictures

Thursday, April 23, 2015
8:18 AM

Figure 1. Temperature sensitive promoter + GFP @ 30, 32, 35, and 37 degrees C

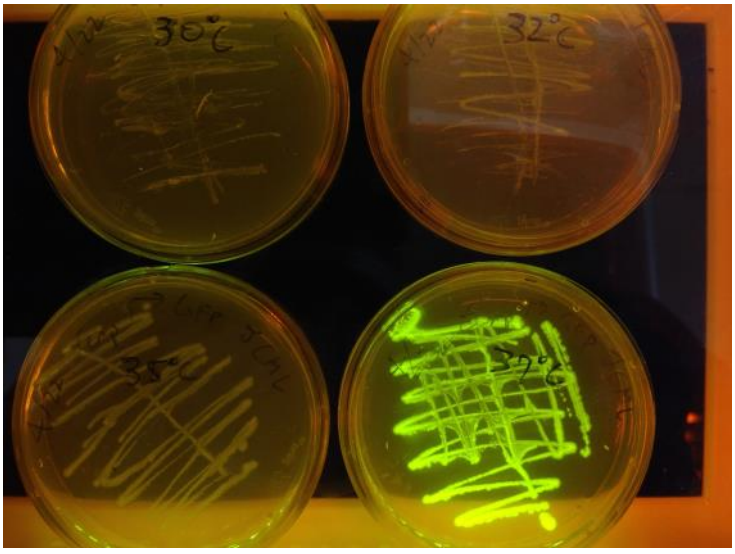
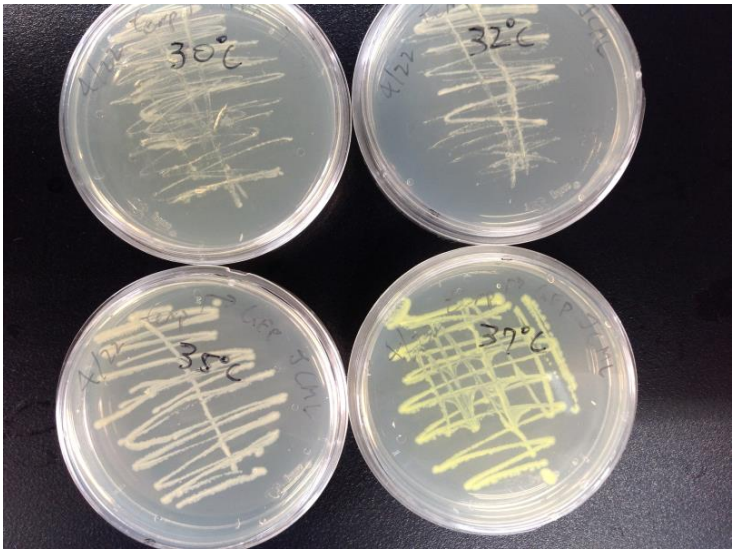


Figure 2. Temperature sensitive promoter + GFP @ 30, 32, 35, and 37 degrees C



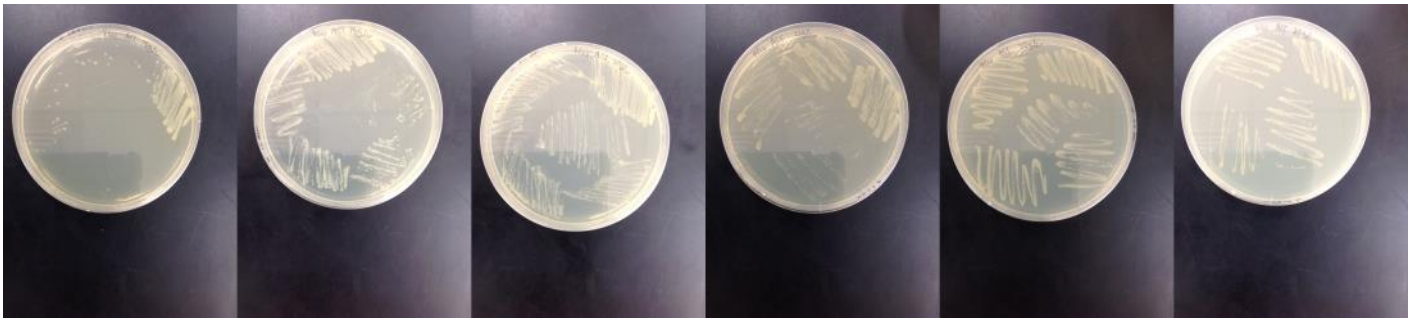


Figure 3 ACT plate 1 restreaks from left to right: 15g/L 17g/L 20g/L 22g/L 25g/L 30g/L

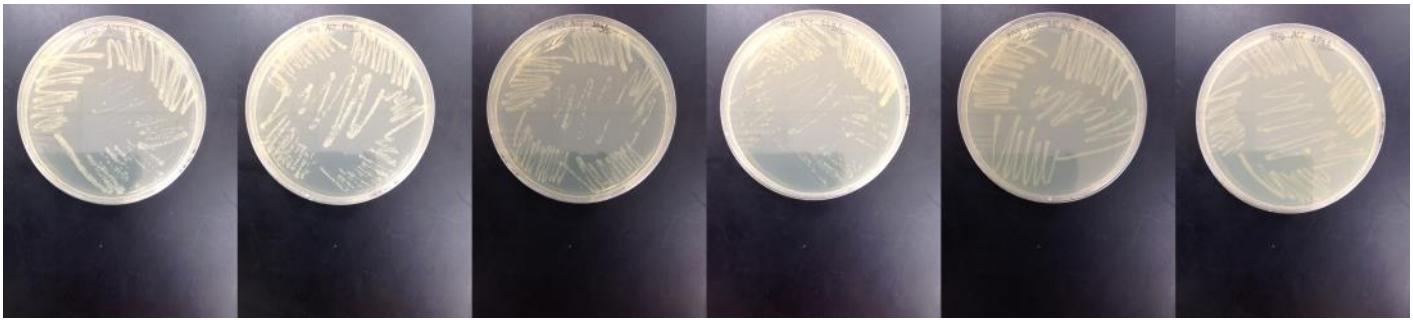


Figure 4 ACT plate 2 restreaks from left to right: 15g/L 17g/L 20g/L 22g/L 25g/L 30g/L

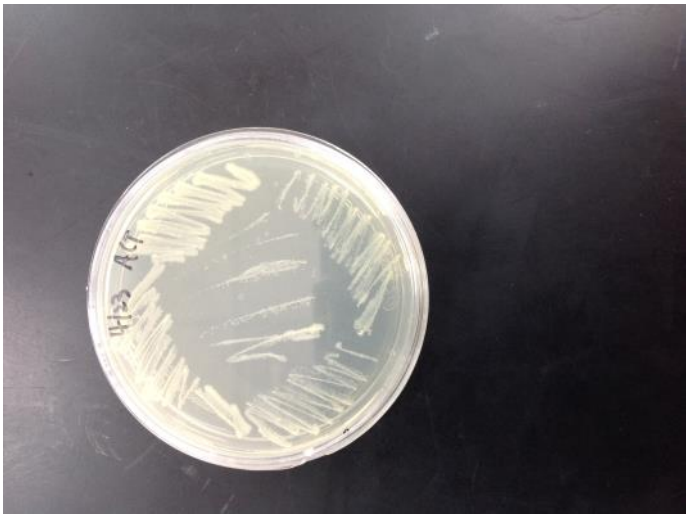


Figure 5 ACT plate 1 restreak

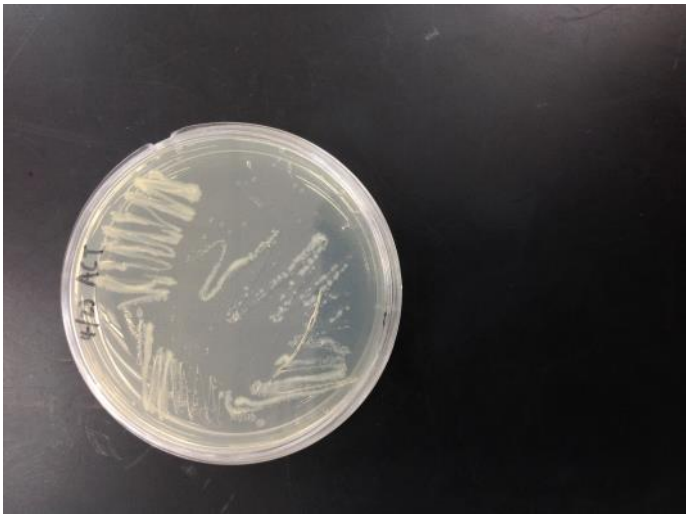


Figure 6 ACT plate 2 restreak

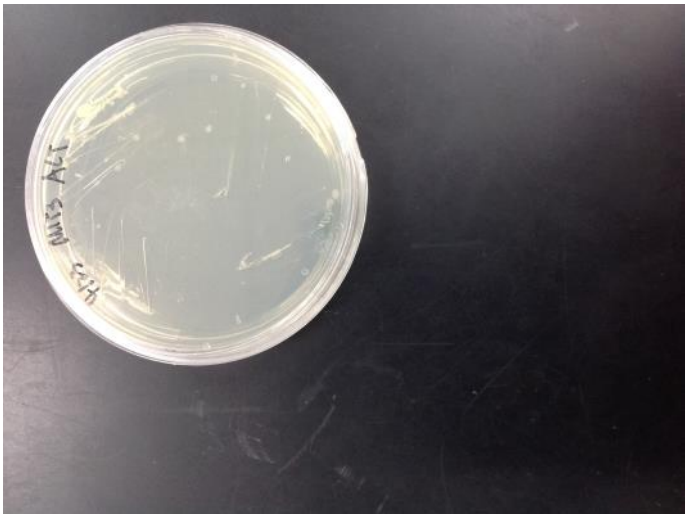


Figure 7 ACT plate 3 restreak

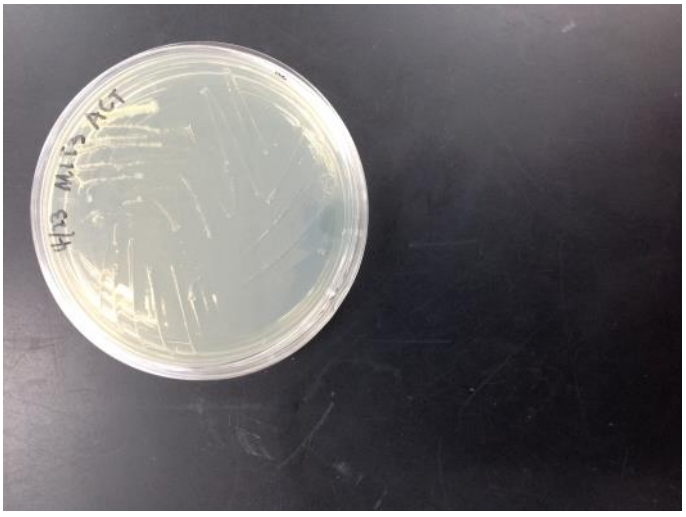


Figure 8 ACT plate 4 restreak

Figure 9 ACT M1T3 B restreak

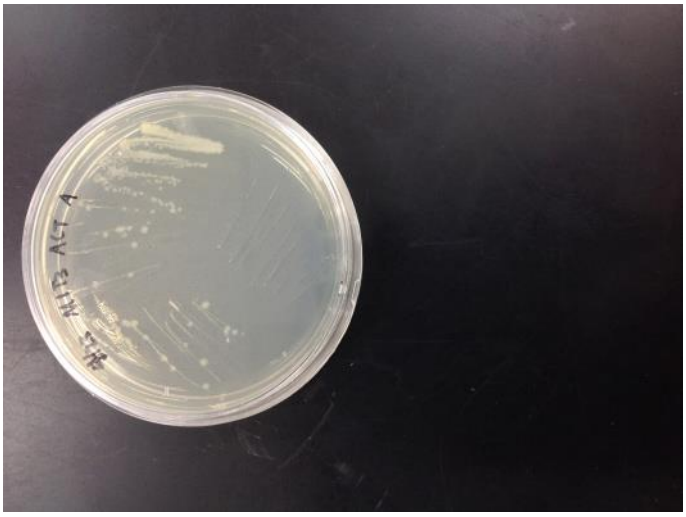


Figure 10 ACT M1T3 A restreak

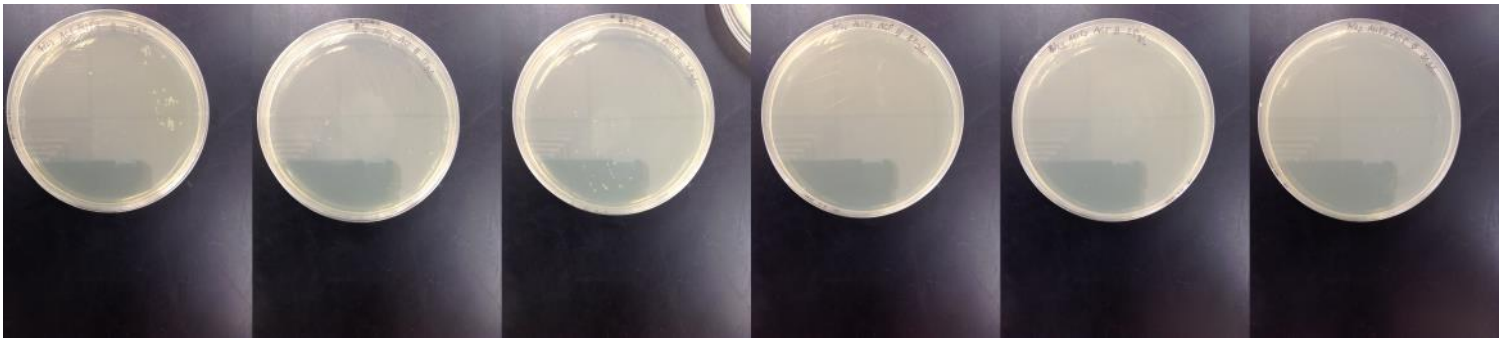


Figure 11 ACT M1T3 B restreaks varying concentrations

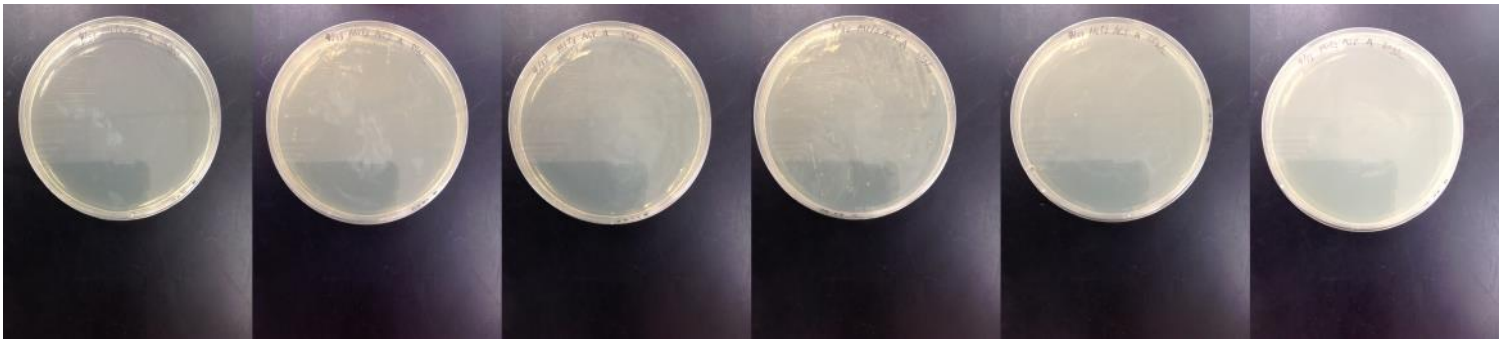


Figure 12 ACT M1T3 A restreaks varying concentrations

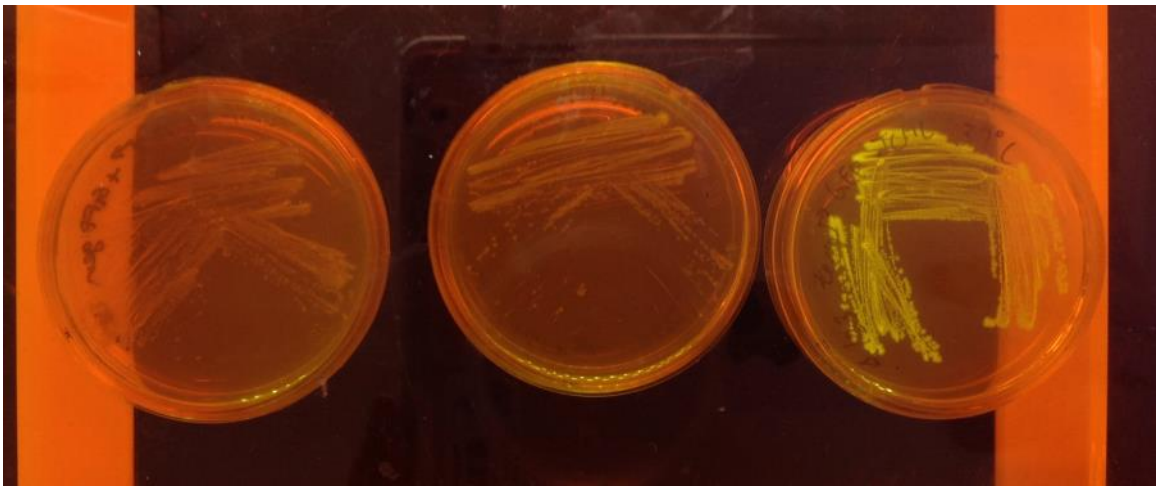
Experiments

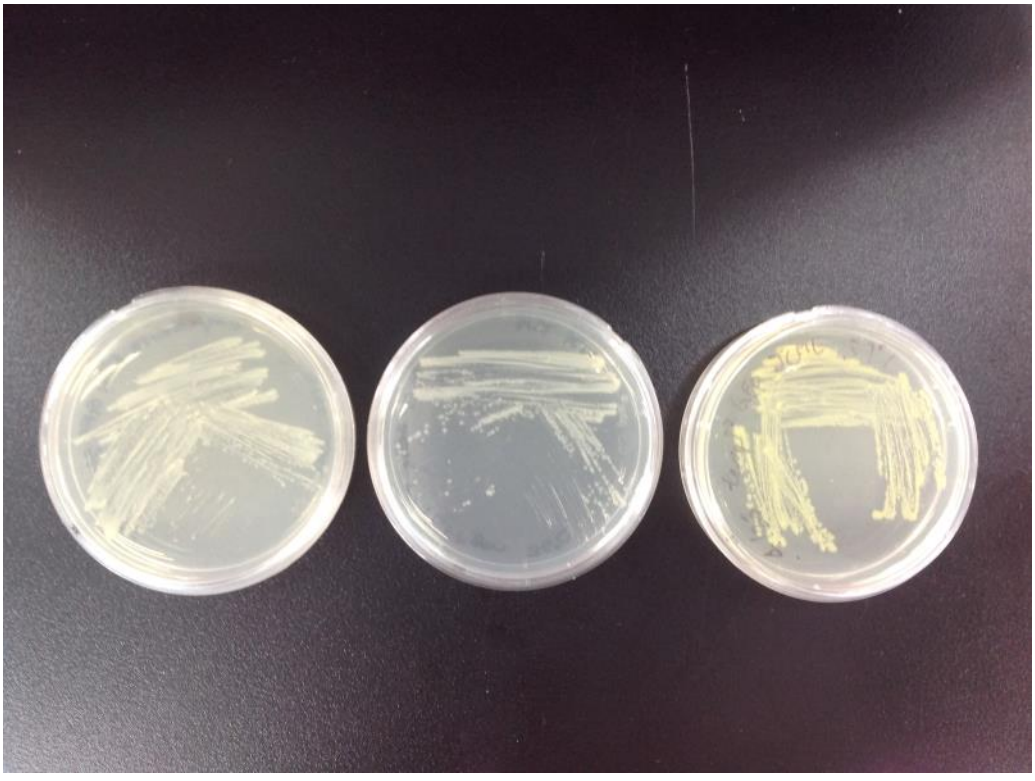
Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	1. Checked temp sens GFP expression	1. See pics 1. Strong at 37, weak at 36 (?), barely at 35	• A gradient?	Either split it down further or try to get liquid culture spectro data
Mini-prep of Temp Sens Promoter	Andrew				
Testing of human body temperature for the temperature sensitive promoter	Paul and Dylan with Dylan being absent				
Testing the new UV promoter	Daphne and Jon				
Temp + RBS	Andrew	•			•
Temp promoter sequencing	Andrew	•			•
Miniprep ACT M1T3 for sequencing	Leon	• Minipreped completed	• 337.0 ng/ul • 471.4 ng/ul	Need sequencing results	Send it out on Monday/Tuesday for sequencing
ACT m1t3 plates and ACT plates	Leon	storage + photograph	storage is nice, bacteria still alive	ready for restreaks and cultures	restreak + culture then sequence

Pictures

Thursday, April 23, 2015
8:18 AM





Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• 4 Liquid Culture tubes		• Preparing for the 3rd prototype	• 4/9 Collect the liquid culture and set-up the membrane tests
Andrew	• Started Temp + RBS experiment • Liquid cultured Temp, Already have RBS digested part(EX) • Re-streaked Temp + GFP at 32 and 37 degrees			• Mini-prep of Temp • Digestion of Temp • Check plates

Experiments

Thursday, April 23, 2015
5:15 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

Andrew	• Mini-prep of Temp
	• Digestion of Temp (ES)
	• Ran gel for Temp
	• Checked plates that were grew on 4/7
	• Re-streaked Temp +GFP, incubated at 37 degrees
		• Plates both grew and both expressed	• New design of way to test Temp+ GFP
			  • Grow at 37 degrees since it grows better
			  • Place plates into different temperatures, RT, 32, 37, 42 degrees
			  • See which ones still glow when RT stops glowing
				• Place Temp+ GFP restreaks into RT, 32, 37, 42 degrees to run experiment next day
				• Ligation and Transformation of Temp + GFP

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• Redo plate membrane test • Prototype for transdermal patches • Two trials • Placing a dialysis tubing on top of an plate filled with LB instead of Agar plate		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body • This time as the 3rd prototype and has to be more sterile • Hopefully combine the benefits of both a plate test and a LB test	• 4/10 The membrane tests
Andrew	• Ligation of Temp and RBS • Transformation of Temp and RBS • Left restreaks into RT, 32, 37 and 42 Degrees, check if RT stops expressing every 4 hours	• RT did not stop expressing	• Keep checking RT plates	• Checking Transformation on 4/10 • Checking RT plate on 4/10

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan	• Collected the membrane test	• Both membrane tests dried out • One leaked	• Never put LB open in incubator, it will dry out	• 4/13 Redo with plate test with agar rand liquid LB membrane test
Andrew	• Transformation of Temp RBS • Did liquid culture for Temp • Temp + GFP experiment	• Transformation of Temp RBS was plated on wrong resistance • RT still glows	• Redo the Temp RBS experiment • Keep Checking	• Mini-prep of Temp

Photos

Tuesday, April 21, 2015
3:15 PM

- Both membrane tests dried out, one leaked
- Figure 1: LB leakage

- Figure 2: Result for the two experiments

Top:

- LB leaked
- Exterior LB dried out in the incubator

Bottom:

- LB didn't leak
- Exterior LB dried out in the incubator

Experiment

Thursday, April 23, 2015
5:31 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Andrew	• Temp + RBS • Temp + GFP testing	• Did mini-prep for Temp • RT still glows	• Have to throw Temp + GFP plates since no one will be in over the weekends	• Digestion of Temp and hopefully ligation + transformation

Summary

Monday, May 04, 2015
8:03 AM

Temperature promoter + RBS
This week, we successfully constructed the temperature promoter + RBS plasmid and ran PCR for it. The results showed that the DNA were the right size.

UV promoter
We've been having trouble with the UV promoter. After testing the new promoters provided by the iGEM headquarter, we found that this promoter also had problems. When we ran gel for the UV promoter, we consistently found bands at 3000 bp instead of at 2000 bp as expected. We also discovered problems when we grew the bacteria on plates. We grew bacteria with the UV promoter on two plates, and one of the plates grew normally white the other plate turned red. We are not sure what is wrong with the promoter, so we have sent it to sequencing.

Temperature sensitive promoter
This week we have been able to conclude that the temperature sensitive promoter is activated at 37 degrees C. This means that when we design our delivery method, we need to consider adding external heat to activate the bacteria as the skin temperature is lower than 37 degree C.

M1T3
We have completed the mutation. However, after sending it to sequencing, we found that the mutation of ACT was unsuccessful. Thus, we are now seeking for an alternative way of accomplishing this point mutation.

M2T4

Prototype
This week we tested our prototype with 0.2 um blue membrane, syringe filter, white filter membrane (size..?), and bandage membrane (positive control). For each design, we did two trials. Even though for each design, one trial was contaminated, the other turned out to filter out bacteria successfully.

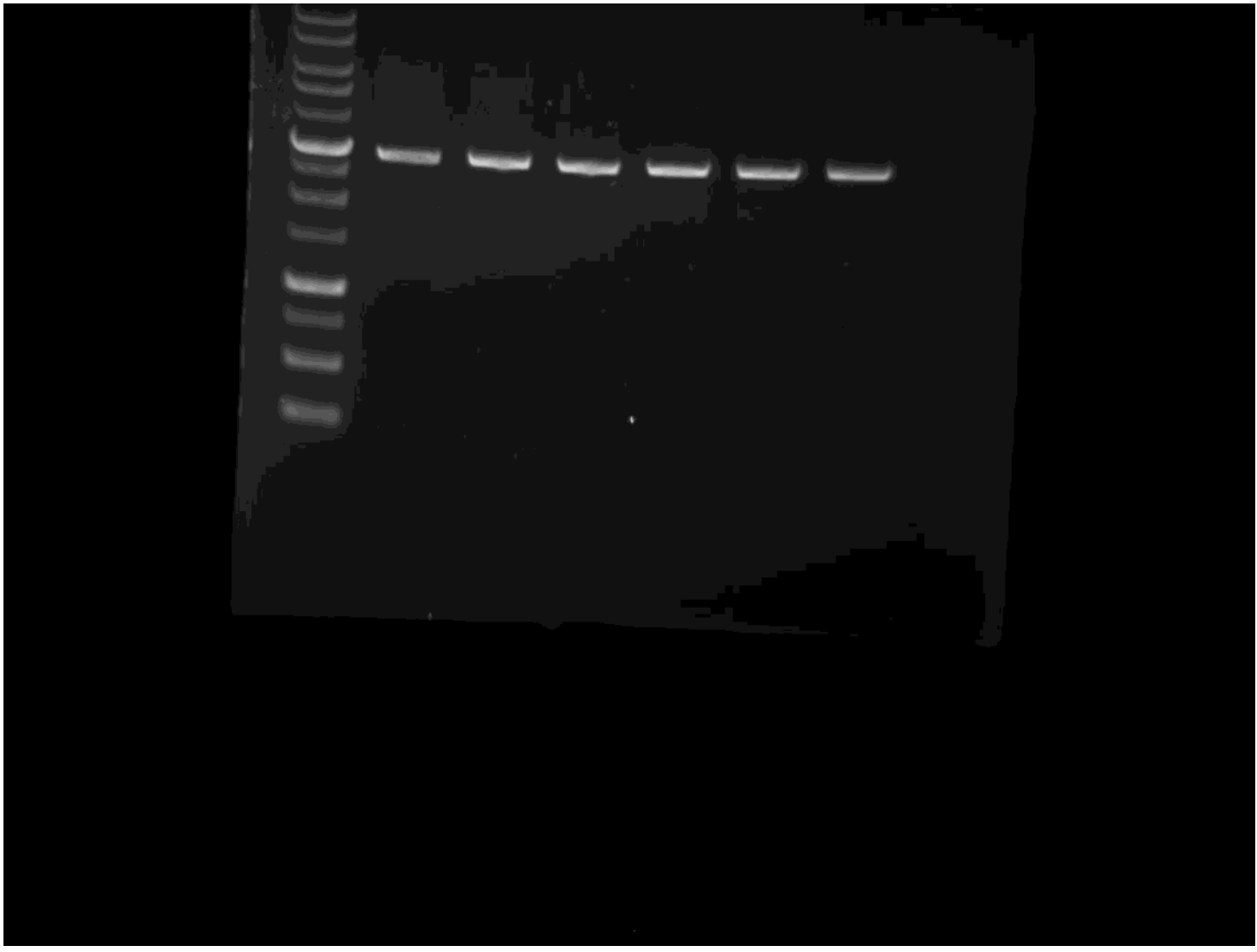
Experiments

Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
UV Promoter	Jon	Liquid Culture for UV Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
Temperature Sensitive Promoter	Jon	Liquid Culture for Temperature Sensitive Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
Temperature Sensitive RBS Promoter	Jon	Liquid Culture for Temperature Sensitive RBS Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
ACT M1T3 A	Jon	Liquid Culture for ACT M1T3 A	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
ACT M1T3 B	Jon	Liquid Culture for ACT M1T3 B	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
UV Promoter (Back insert with GFP)	Daphne	Digestion for UV promoter, cut with S and P	Digestion and gel check completed but it came out the wrong size on the gel. The bands were at near 3,000 bps instead of the expected 2,100 bps of UV promoter.	Redo digestion again tomorrow and cut at E and P.	Digestion
Temp RBS construct	Andrew	Gel check Of Temp RBS PCR	Size is right		Liquid culture and miniprep
Temp RBS construct	Andrew	PCR 2 more colonies Restreaked all 3 colonies used for PCR			Do gel check and check restreaks
Temperature promoter	Paul and Dylan (Dylan was absent so it was Evonne who served as the recording partner)	Collecting body temperature data recordings from members of the TAS student and faculty body using Mr. Tsao's IR laser temperature sensor.	Successfully recorded the sufficient amount of data sets from the TAS public. We collected 20 data points from the female members of the TAS community while we collected 14 data points from the male members of the TAS public	Successful collection of the data points, now we will process the data points on the shared Google Doc spreadsheet with the rest of the team	Data processing and possible experimental work

Pictures

Thursday, April 23, 2015
8:18 AM



Experiments

Tuesday, April 28, 2015
2:39 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT M2T3	**Leon**	**PCR ACT M2T3 Standard protocol**	**Notsure yet**	**None yet**	**Dpn1 + transform**
ACT M1T3	**Leon**	**Miniprep ACT M1T3 A1 A2 B1 B2**	**Good concentrations**	**Should sequence them next time**	**Sequence**
NEW UV Promoter testing	**Daphne+ Phillip**	**Digestion (at E and P) and gel check**	**The bands were still at 3 kb.** **Please refer to figure 1 under the pictures tab.**	**Restreak to send for sequencing.** **The results make it very confusing because we are not expecting the**	**miniprep**
		1.		**New**	

Pictures

Tuesday, April 28, 2015
3:12 PM

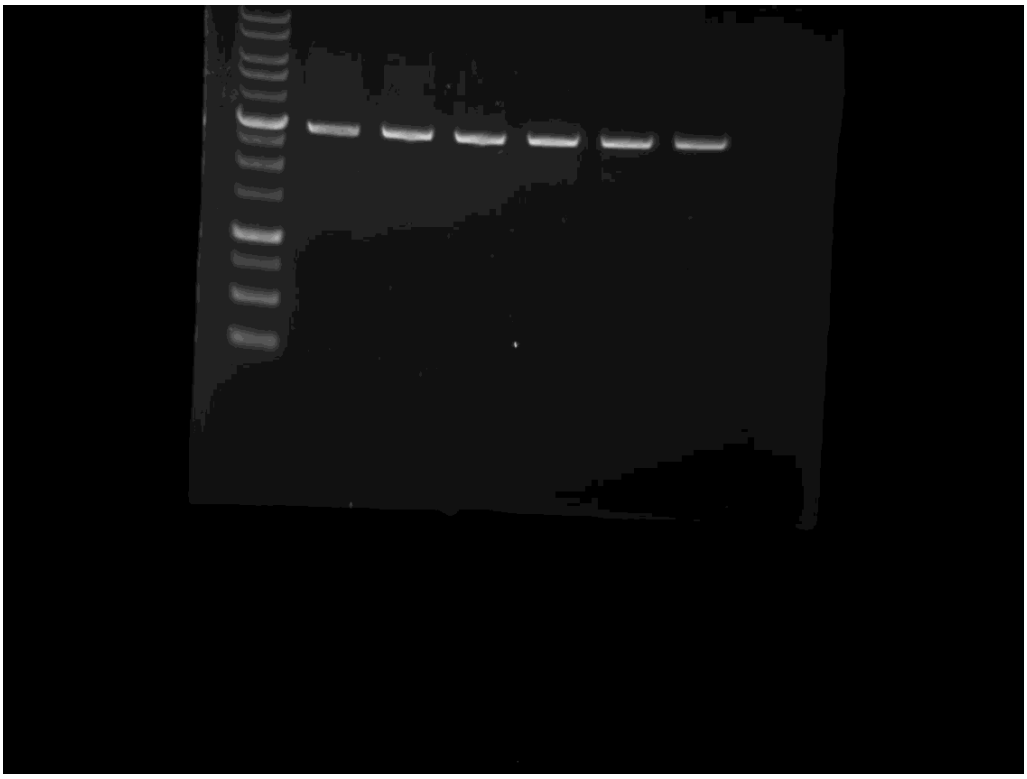


Figure #1: The gel check for the UV promoter cut at EP is shown here to be around a length of 3k.

Experiments

Tuesday, April 28, 2015
2:39 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT M2T3	**Leon**	**Dpn1 + transform (figure 2)**	**Not sure yet**	**None yet**	
ACT M1T3	**Leon+ Phillip**	**Restreaked on to plates** **Grew cultures for plate a and plate b (figure 3,4)**	**Gonna check tomorrow**	**They can be found in the Mutagenesis box.** **They were sent for sent for sequencing but we didn't have enough concentration.** **We now learn that sequencing can take liquid culture and bacteria**	
ACT M2T4	Leon	Setup PCR for ACT M2T4 New PCR program test 5 cycles of reduced heat 30 cycles of normal heat Potential problems reduced heat by too much?	None yet	We'll see how we will edit the PCR program	Should definitely run gel and dpn1 + transform
NEW UV Promoter testing	**Daphne**	**Streak from** **MIT3 ACT B** **MIT3 ACT A** **Bba_1765001A** **BBA_1765001B** **Temp + RBS**			**Liquid culture**
Temperature promoter data processing and lab maintenance	**Paul**	**I refilled the pipette boxes that have been running low on pipettes, and I also looked at the data tables for the data collected during our field testing of people's body temperatures.**	**Boxes have been refilled and are going under the decontamination processes of autoclaving. I am also working on the data processing**	**Lab is now in a more desirable condition since the pipette boxes have now been refilled and I have started the data processing procedure for the body temperature community data points**	**Continue the data processing process and also see if the lab needs any maintenance or experimental work that needs to get worked on**
UV Promoter Testing	**Jonathan**	**Miniprep 5mL each.** **1 for sequencing and one for further experimentation**	**Concentration:** **For sequencing** **For further experimentation:**	**F9 Leon's Box you can find it :D** **Right next to it is**	
Andrew	Temperature Promoter+ RBS	Ran gel check for the PCR results for Temp Prom+RBS. Additionally, did miniprep for the Temp Prom+ RBS.			
Joseph, Monica	Temperature promoter + GFP Temperature promoter alone	Starting miniprep by liquid cultures			Follow up with miniprep

Pictures

Tuesday, April 28, 2015
3:12 PM

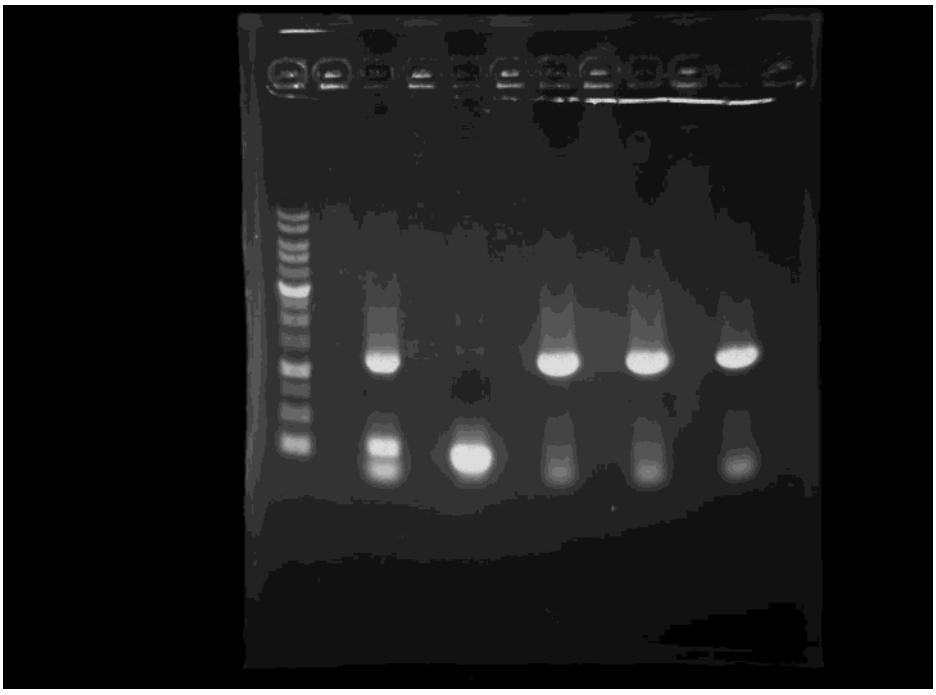


Fig. 1 this shows the gel check for Temperature+RBS result from the PCR.
This is the order of the gel starting from the left to the right. 1kb ladder, Positive control (pLac+GFP Gen), negative control (RBS vector), And everything else is Temperature Promoter + RBS.
Please refer to experiments to see conclusion

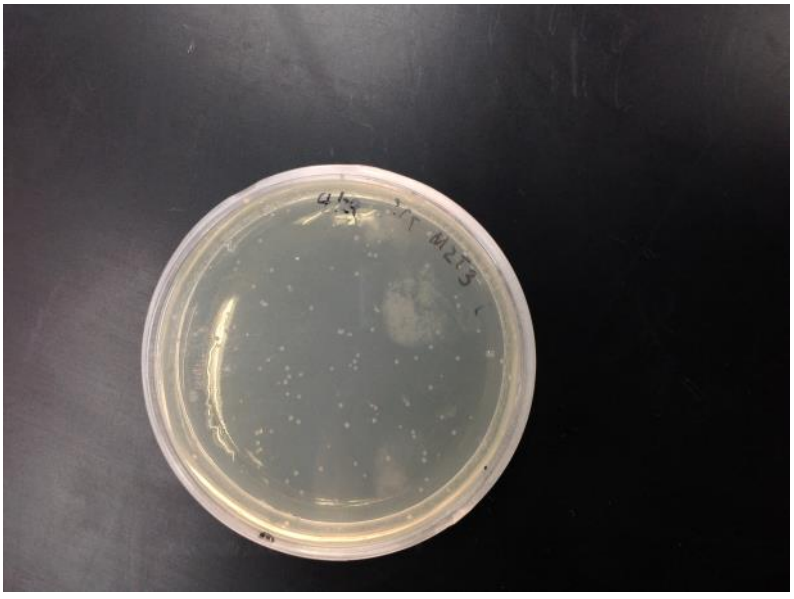


Figure 2 ACT M2T3 transformation needs to be sequenced for results

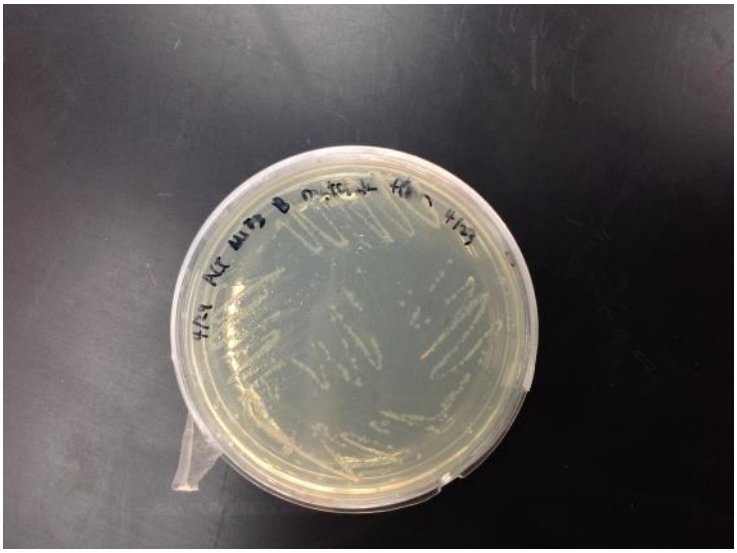


Figure 2 ACT M1T3 B restreak

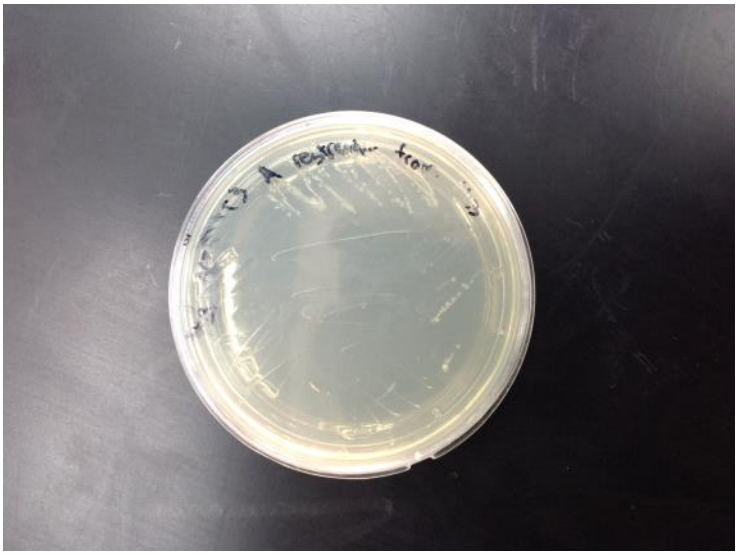


Figure 3 ACT M1T3 A restreak

Sequencing Protocol and Notes

Wednesday, April 29, 2015
2:49 PM

Any questions, ask Phillip

To get sequencing results

Experiments

Wednesday, April 29, 2015
2:46 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
M2T4	**Leon+ Phillip**	**Took the PCR product, digested then transformed**	**Both grew, negative control too**	**Can't use this stuff troubleshoot sent out for other companies to do for us**	
M1T3	**Leon**	**Miniprep + sequencing step**	**2 tubes at 400,500 ng/ul 4 tubes at 100~ ng/ul**	**Not sure if they are mutated**	**Wait for sequencing results**
I765001 UV	**Leon**	**3 in 1**	**One plate was red one was white labeled individually**	**Not sure why one is red and one is white (Figure 1,2)**	Wait for sequencing
ACT M1T3, ACT M1T2, ACT	Leon	Restreaks	Fun	They shall grow Figure 5,6,7,8, 9 Figure 10,11 Figure 12	Save em'
Miniprep	Jo and Monica	Miniprep from last time	More fun		Awaiting further orders from central *What are the concentrations? -central* Pretty low - will try re-miniprepping
UV promoter	Monica	Miniprep 2 tubes of 5 mL UV promoter + RPF (white) and 1 tube of 5 mL UV promoter + RPF (red)	White = 100.1 ng/ul Red = 495.1 ng/ul		Send to sequencing

Pictures

Wednesday, April 29, 2015
3:24 PM

Figure 1 I765001 white

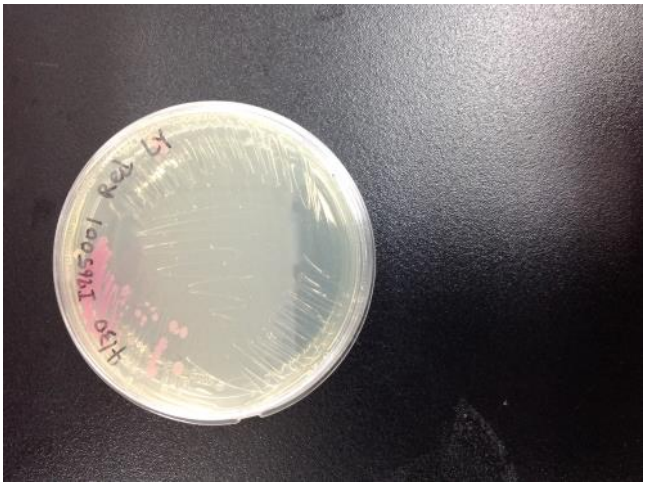


Figure 2 I765001 Red

Figure 3 ACT M2T4

Figure 4 ACT M2T4 Negative (failed)

Figure 5,6,7,8 left to right: ACT M2T3 A, B, C, D

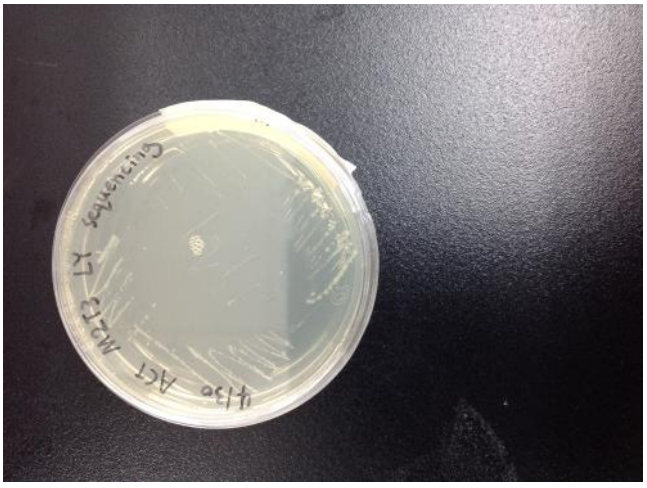


Figure 9 ACT M2T3 sequencing restreak

Figure 10 ACT M1T2 restreak from transformation salvage plate 1

Figure 11 ACT M1T2 restreak from transformation salvage plate 2

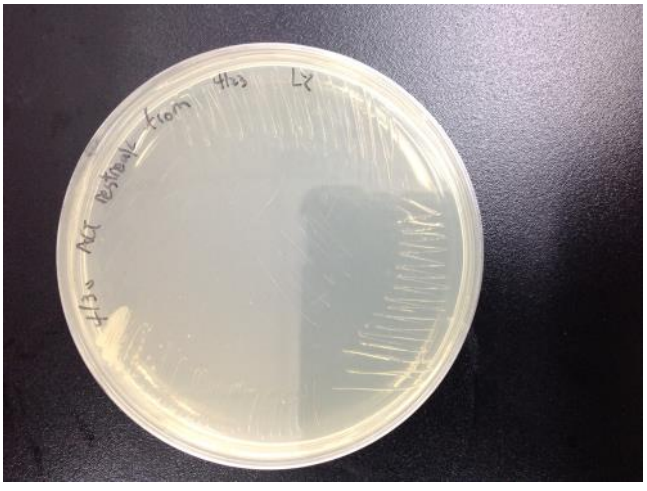


Figure 12 ACT restreak from restreak (needs one more restreak)